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Analysis of *Bacillus Globigii* Spores Using the BioDetector

by

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Abstract

The Canadian Forces have a mandate to be able to operate under the threat of chemical or biological attack. An automated immunoassay instrument capable of providing rapid identification of biological agents was used to analyse laboratory and field trial samples containing the field trial simulant *Bacillus globigii* (BG) spores. The BioDetector instrument was developed for US Army Edgewood Research Development and Engineering Center by Environmental Technologies Group. It was an automated version of the Threshold system (Molecular Device Corp) employing filtration-capture immunoassays and a light-addressable potentiometric sensor (LAPS). The experiments presented herein demonstrated that the instrument was capable of determining the presence of BG spores in the liquid laboratory and field trials samples. The time required to carry out the assays was 15 min. The lower limits of detection were in the range of 10 - 30 ng/mL. The instrument software provided a four level qualitative alarm for each sample: negative, low, medium and high. The principal limitations of the instrument were its large size and complicated fluidic network.

Executive Summary

Title:

Analysis of *Bacillus Globigii* Spores Using the BioDetector, William E. Lee and H. Gail Thompson, Technical Memorandum 1999-154.

Introduction:

The Canadian Forces have a mandate to be able to operate under the threat of chemical or biological attack. In order to provide effective protection and therapy against biological warfare (BW) agents, reliable means of detection and identification of these threats are required. Biosensors, coupled with advances in immunoassays, can provide detection and identification systems for biological threat agents. An automated immunoassay instrument capable of providing rapid identification of biological agents was used to analyse laboratory and field trial samples containing the field trial simulant *Bacillus globigii* (BG) spores.

The BioDetector instrument was developed for US Army Edgewood Research Development and Engineering Center by Environmental Technologies Group. It was an automated version of the Threshold system (Molecular Device Corp) which employs filtration-capture immunoassays and a light-addressable potentiometric sensor (LAPS). This work demonstrated that the instrument was capable of determining the presence of BG spores in liquid laboratory-prepared samples and field trials samples. The time required to carry out the assays was 15 min. The instrument software provided a four level qualitative alarm for each sample: negative, low, medium and high. The principal limitations of the instrument were its large size and complicated fluidic network.

Results:

The BioDetector instrument was employed to monitor liquid samples for the presence of BG. From the analysis of standard calibration curves of laboratory samples the lower limits of detection (LOD) were determined to be in the range of 10 - 30 ng/mL. These values were comparable, though slightly higher than the LOD obtained for BG on the manual Threshold system.

The field trial samples used in this work were collected at Dugway Proving Ground, Utah at the Joint Field Trials in September 1997 using three independent aerosol collectors. The BioDetector gave positive alarms for each trial in which BG was disseminated and did not give false positive alarms for trials in which another simulant, *Erwinia herbicola*, was used. Correlation between the responses of the collectors was observed.

The operation of the instrument required low effort and skill on the part of the operator compared to the manual version of the LAPS sensor (Threshold system). Liquid samples contained in plastic culture tubes were inserted into the sample compartment. The assay process was automated thereafter. When the instrument was loaded with the reagent tray and the membrane cassette it was capable of performing 35 assays (1 mission = 35 assays). Thirteen missions were attempted; four resulted in failure that required reinitializing of the instrument. Three of the failures were a result of the cassette running out of membrane and failing to advise the operator. This problem was subsequently overcome by the manufacturer rewriting the control software.

Significance of Results:

The instrument was able to take a sample, aliquot it to eight independent channels and parallel process each channel by immunoassay, and provide a four-level qualitative analysis within fifteen minutes. The version of the BD instrument that was used at DRES was successful in its assay performance. The limitations to the design are the relatively large size of the instrument and the complex fluidics. The filtration-separation step, in which the immune complexes are immobilized on the nitrocellulose membrane, requires a bulky cassette to hold the membrane and a mechanical tape drive to advance it. A fresh membrane frame is required for each test. Although the tape drive functioned throughout the experiments, being mechanical, it was succeptible to breakdown.

The total assay time was 15 min of which only 3.5 min was allotted to immunochemistry. The remainder was taken up by transporting fluids, filtration, washing and membrane reading. New generation identification instruments for biological agents would ideally be smaller, have fewer mechanical subsystems and perform assays in a shorter time period. Analytical devices based on microchip platforms would provide many desirable features in new generation instrumentation.

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Introduction

Canadian Forces have a mandate to be able to operate under the threat of chemical and biological attack. In order to provide effective protection and therapy against biological warfare (BW) agents, reliable means of detection and identification of these threats are required. The ideal instrument that would fulfill this need is one that is compact, automated, rapid, sensitive and capable of identifying a wide range of biological agents. Biosensors, coupled with advances in immunoassays, can provide detection and identification systems for biological threat agents.

A silicon sensor-based immunoassay for the identification of biological agents in liquid samples that has been useful for defence-related bioanalysis is the Threshold system manufactured by Molecular Devices Corp (1). This unit employs "sandwich" immunoassays using a light-addressable potentiometric sensor (LAPS) that monitors pH change (2). Reagent antibodies and the antigens are incubated together in homogeneous phase in a single step. The resulting sandwich immune complexes are filtered through biotin-embedded nitrocellulose membrane and immobilized on the membrane by means of biotin-streptavidin interactions. A urease-conjugated second antibody is subsequently reacted with the immune complex on the membrane. The membrane is placed in contact with the LAPS in the presence of urea-containing buffer. Hydrolysis of urea and the consequent rate of change of pH with respect to time at the surface of the silicon sensor is monitored by the change of the surface potential as μV/sec (Figure 1). The Threshold system has been employed in immunoassays for proteins, viruses and bacteria (3-8). As well, the system has also been used for gene probe assays and for detection of organophosphorus compounds (10).

An automated version of the Threshold system, the BioDetector (BD), was developed by US Army Edgewood Research Development and Engineering Center in collaboration with Canada and United Kingdom under the MOU for CB Defence. The BD is the final version of the development project. The experiments on the BD were carried out at DRES. Earlier versions of the BD were also tested at DRES (16).

In the operation of the BD, a liquid sample (1 mL or greater) contained in a plastic culture tube was placed in the sample carrier. The immunoassay analysis was automated from that point onward. The sample was aliquoted to eight channels; each channel was capable of carrying out a separate immunoassay in parallel. After the assay was completed, the system was automatically cleaned and advanced and made ready for the next sample. In cases where a high alarm was

registered by the BD, the surface of the reader-chip was manually cleaned with a rubber swab. In this work, a single analyte, a spore preparation of *Bacillus globigii* (BG), was used. All eight channels were supplied with the same immuno-reagents for BG. The BD system was assessed for performance, reliability, variation, and sensitivity. *Bacillus globigii* was chosen as the analyte for this work because it is one of the field trial simulants used at Dugway Proving Ground and at Defence Research Establishment Suffield. The BG samples used in the assays were prepared in the laboratory or obtained from field trials at Dugway. Previous studies on BG have described the immunoassay characteristics of the spore preparation in Threshold assays (11).

Materials and Methods

The BD was manufactured by Environmental Technologies Group (ETG) of Baltimore, MD, under contract from the US Army Edgewood Research Development and Engineering Center (Aberdeen Proving Ground, MD). The system was intended for use inside a shelter or mobile truck-based platform and operates on AC or DC power. It is considered a two-man portable system weighing 55 kg and measuring 55 cm in width, 55 cm in height, and 45 cm in depth (Figure 2). The instrument was designed for operation between 10 and 27 °C and storage between -46 and 71 °C.

The reagent trays, containing sealed vials of lyophilized biotin and fluorescein labelled antibodies against BG, anti-fluorescein urease, and negative control reagent, were supplied by ETG along with dilution buffer and wash buffer. The caps of the sealed vials were removed prior to use and the reagents were reconstituted by the instrument where required. The reagents in the tray were sufficient to run approximately 35 BG samples (1 mission = 35 samples). The reagent tray module is shown in Figure 3. Cleandown trays were also supplied with the instrument and were used after the contents of the reagent tray were consumed and the mission completed. Biotin-embedded nitrocellulose membrane was supplied on a continuous tape in cassette form (Figure 4). The cassette was replaced by the operator as prompted by the liquid crystal display on the instrument. An exposed view of the BD with the reagent tray and and membrane cassette installed is shown in Figure 5.

BG was obtained as a lyophilized powder from the Baker Laboratory, Dugway Proving Ground, Utah and reconstituted in distilled water to the appropriate concentrations for the laboratory samples. Field trial samples were collected during the Joint Field Trials (JFT) IV in September 1997 at Dugway Proving Ground and were kept frozen at -70 °C until use. Three aerosol collectors were

used in the field trials: a large volume air sampler (LVAS) located in the mobile laboratory trailer (12), a LVAS designated V97, and an XMX aerosol collector (Dycor Industrial Research, Edmonton AB) located nearby on the test grid with the CIBADS CB sentry.

An aliquot (1 mL volume) of each sample or standard was contained in a small polystyrene culture tube which was inserted in the sample tube carrier of the BD device and locked into place. At this point, the 15 minute BG assay commenced automatically. An assay timing diagram is shown in Figure 6. On completion of the assay, the sample tube was removed and the next sample was inserted in the sample-tube carrier of the BD device. The instrument automatically performed negative control assays after every five identification assays.

Results

Standard curves for BG were run for each mission on the BD, either at the beginning of a set of analyses or at the end. Six standard curves were generated and the limits of detection (LODs) were calculated from the data (examples are shown in Figures 7 and 8). The standard curves were generated by manual entry into commercial software of the downloaded BD data. The LOD was defined as the intersection of the calibration curve with the negative control plus two standard deviations on the negative control. For determination of the LODs, assay buffer was used as the negative control. The LODs are compiled in Table I. Although the measured LODs showed some variation, the values lay in the range of 10 - 30 ng/mL. The major determining factor for the calculated LODs was the standard deviation on the negative control. Previous BG assays performed on the Threshold Unit with antibody reagents prepared in-house gave LODs of 3 - 6 ng/mL (11). Thus the LOD data determined on the BD was comparable, although higher due to the greater standard deviations on the negative control. The concentration range of the standards was large: 0 - 5000 ng/mL as shown in Figure 8. Calculation of the LODs was accomplished using the lower concentration portions of the standard curves. Concentrations of unknown samples were determined by interpolation from the standard curve in the appropriate range. In the standard curves, the signal response, [slope of calibration curve, i.e., $(\Delta\ \mu V\ sec^{-1})/(\Delta ng\ mL^{-1})$], decreased with increasing concentration of BG. This was attributable to a combination of the inherent decrease in sensitivity at higher concentration plus the possible onset of a "prozone" or "hook" effect (13, 14). In homogeneous single phase assays, "sandwich formation" (as depicted by Figure 1) is proportional to analyte concentration when the antibody reagents are in large excess to the antigen and thus the

probability of making a two-antibody-antigen sandwich for each antigen present is high. As the antigen concentration increases so does the probability of making non-signal generating single antibody-analyte complexes (i.e., open-face sandwiches). However, the prozone effect did not appear to be an overriding problem; positive signal response with increasing concentration was observed throughout at all concentrations used. With the Threshold system, the standard procedure for overcoming possible prozone effects was to dilute the sample. In field trials with the Threshold, if low (or no) signals were obtained in instances where the operator suspects the presence of analyte (that is, the first line detection devices indicate a positive in the aerosol monitoring), the sample can be run again at 1/100 or 1/1000 dilution. If the initial concentration of antigen is too high for the assay reagents, then "diluting out" the prozone effect will cause an increase in signal.

The instrument collected data for eight channels per run. A four-level alarm reading was displayed as negative, low, medium or high. The data could also be given in terms of the actual LAPS sensor signals, μV/sec. The instrument stored data for 100 runs in memory.

Field trial samples were obtained from three aerosol collectors. The LVAS was located in the mobile trailer laboratory; and the V97 and the XMX were located at the sentry site near the trailer. The positioning of the collectors was such that they were in line approximately perpendicular to the path of the simulant clouds. The distance between the trailer and the sentry site was about 50 m; and so the collectors sampled similar (although not identical) aerosol concentrations of BG. The operation of the collectors differed. The LVAS collected 1000 L air/min and delivered samples in continuous flow at at rate of 1 mL/min for about 10 min. The XMX collected 800-1000 L air/min into a fixed sample volume of 20 mL. Each field trial sample represents a ten minute collection of the simulant cloud as it passed the collector. The collectors were triggered by an aerosol particle detector located in the mobile laboratory. Between trials, the air was sampled for ambient aerosol particles using the LVAS in the mobile laboratory.

The concentration results of the BD response for the field trial samples (Tables II & III) are averages of either 7 or 8 channels with corresponding standard deviations. Low and high concentration response (standard) curves were both used in the quantitation of the JFT IV samples, depending on the signal strength of the trial sample. Examples of low and high concentration calibration curves of BG are shown in Figures 7 and 8. These results were calculated after the testing had been completed and the data were downloaded. The alarm responses shown in the tables were produced by the BD in real time at the end of the 15 minute sample processing. Each channel was

able to produce an alarm response independently. The qualitative values shown in the table were the average four-level of all the channels in the trial.

Table II shows the BD response for the the aerosol samples collected in the mobile laboratory using the LVAS for the trials where BG was dispersed. Also given in the table are the BD responses to the environmental backgrounds that were collected between runs. In each case where BG was intentionally dispersed and collected, the BD gave a positive indication. There were no false alarms by the BD when the agent sprayed was the field trial simulant bacteria *Erwinia herbicola*. During the monitoring of the environmental background prior to the BG field trial dispersals, there were several alarms registered on the BD for BG. These alarms appeared to be actual positive indications for BG and not the result of contamination of the collector or the BD sensor, or faulty immunochemistry. The area at Dugway where the trials were conducted has considerable accumulated amounts of ambient BG.

Table III shows the BD response for BG collected at the CB sentry site with the LVAS (V97) and the XMX aerosol sampler. These were operated in a similar manner to the LVAS; the trigger to initiate collection and the sampling periods were the same. The responses of the BD alarm for the V97 and XMX were qualitatively similar to that of the LVAS. There were a few instances where positive alarms were indicated by the BD for the LVAS samples but not for the V97 or XMX. This can be attributed more to differences in airborne BG concentrations rather than differences in BD response. There were also four instances where there was a low level alarm for the V97 and no alarm for the XMX (Table III). These latter discrepancies were more likely due to differences in the aerosol collection process (position of cloud, collector efficiency and dilution) than to BD variability. The BD signals (μV/sec) and the S/N ratios were generally higher for the V97 than the XMX; that is, there was probably more BG per sample volume in the V97 samples.

Operational Review

The automated BD was operated at DRES as a stand alone device with fixed assay parameters such as timing, reagent concentrations, and reagent and sample volumes set by the manufacturer. The instrument could be interfaced with a PC computer for downloading the signals generated by the sensor chip. The resident software in the BD that controlled the timing sequences and reaction volumes of the assay was not accessible to the operator.

The alarm criteria of the BD were independent of the aquisition of standard curves and LOD data, rather based on the magnitude of the signal above the negative control. For the BG assay, a signal greater than the negative control plus 115 μV/sec was indicated with a low alarm. Signals greater than the negative control plus 1000 μV/sec and 2000 μV/sec were medium and high alarms, respectively. The BD automatically performed a negative control after every five identification assays in order to monitor the drift in the output signals from the LAPS. For operation of the BD, it was not necessary carry out a standard curve analysis. The reagent trays provided all reagents required, including buffers and negative controls.

Pre-analysis set-up by the operator consisted of entering the current date, identifying the contents of the assay tray by means of a bar code reader that plugged into the BD, loading the tray in the drawer, and inserting a cassette containing biotin-embedded nitrocellulose tape. The liquid crystal display then prompted the operator as to whether or not the reagents in the tray needed to be reconstituted. After reconstitution of the reagents, the instrument initiated the priming of the various fluid lines and then performed a negative control assay. If all spots were below a pre-set threshold level (which they always were during the time of operation of the BD), analyses of the samples could proceed. The instrument software had a preset level for the negative control that was not accessible to the user; it was in the approximate range of 125 - 150 μV/sec. For the most part, the negative controls were generally 50 - 100 μV/sec (for example see Figure 7). Thus, using calibration data from Figure 5, we can calculate that a sample of 50 - 100 ng/mL would correspond a low level alarm.

The total time of an assay is slightly less than 15 minutes from insertion of the sample to the display of the results. This is approximately 5 minutes less than the standard Threshold assay and is due mainly to the decreased incubation time (12). A 10 minute incubation time is used for most of the standard Threshold assays, whereas the BD only incubates a sample with reagent for 3.5 minutes (timing sequence Figure 6).

In comparison with the Threshold, the operation of the BD is simplified. While both instruments require a high logistic load, the Threshold is high skill and operator intensive, whereas the BD is low skill and effort, requiring only that the sample tubes be inserted into the sample carrier. The reagents for the BD were prepared to accommodate a wider (higher) range of BG concentrations than that usually employed for the standard Threshold assays. For the Threshold assays, the reagent concentrations were optimized for sensitivity in the low analyte concentration regime. The higher

concentration of reagents used in the BD assays allowed for an extended analyte range (without succumbing to prozone or hook effects).

The testing showed that on a single membrane frame, the spot-to-spot coefficient of variation on the BD was in the order of 15% to 35% with the higher values more common. The Threshold generally produces coefficients of variation among spots of 5% or less (5,6). The lesser variation in the Threshold can be attributed to longer incubation times, greater accuracy of fluid delivery and better mixing of reagents by a skilled operator than by the fluidic processing systems in the BD.

Thirteen missions were attempted; four resulted in operational failures that required reinitialization of the instrument. On three separate occasions, the unit failed to warn the operator that the tape had expired. These events necessitated having to power down the instrument and abandon the mission. With the software configuration that was installed there was no way for the operator to bypass the priming stage on powering up and since this stage effectively consumed reagents equivalent to 30 runs, the reagent tray had to be changed, even if only a few assays had been completed. Also, after priming the lines, the assay count is reset to zero so that even though there may be several more assays possible, there would be no reliable way of telling when the reagents were spent.

During the assessment, a problem occurred when the operator failed to remove two of the caps from the antibody vials in the reagent tray. This could have resulted in bent reagent probes, although fortunately, in this case, the probes completely pierced the rubber septums on the vial and no damage to the probes was observed. However, results from this channel (#8) were erratic for much of the testing, possibly due to pieces of rubber being lodged in the two probes. Signals generated from this channel were ignored until consistency with the other seven spots was restored.

Discussion and Conclusions

The BD was easy to use and its operational configuration virtually eliminates the possibility of technical errors in the assays themselves, particularly when testing for an array of agents (up to eight). However, in its simplicity and ease of use, operational malfunction can arise (e.g. the cassette runs out of membrane tape and fails to warn) and cannot be overridden without undergoing a complete power down. While this prevents an inexperienced user from making mistakes, in certain situations such as when the unit failed to warn of tape expiration, it would have been more convenient to replace the tape cassette without having had to shut off the instrument, replace the

reagent tray, and begin again with a new mission. These problems were minor in nature and were in fact subsequently corrected by software modifications. Another software modification, made after this work was completed, was in the "power-up reprime" routine. After modification the priming consumed six runs worth of reagents rather than thirty with previous software.

The software that was provided with the instrument, only allowed for the downloading of data from the BD to a computer. The manufacturer had to be contacted if changes to the alarm criteria or the designation of agents were desired. Changes in alarm criteria might be necessary to account for the presence of high background or interferents. Also, agents other than those predesignated cannot be assigned a channel. Although it is possible to load the reagent trays with custom reagent for other immunoassay antigens, that is, analyte antigens other than the standard set, the instrument software did not permit name changes to the channels, e.g., channel #1 would always be designated as anthrax. The software was purposely made inaccessible. ETG and the US Army designed the BD so that the soldier in the field would not be able to modify the operation parameters.

Overall the instrument was able to take a sample, aliquot it to eight independent channels and parallel process each channel by immunoassay, and provide a four-level qualitative analysis within fifteen minutes. The version of the BD instrument that was used at DRES was successful in its assay performance. The limitations to the design are the relatively large size of the instrument and the complex fluidics. The filtration-separation step, in which the immune complexes are immobilized on the nitrocellulose membrane, requires a bulky cassette to hold the membrane and a mechanical tape drive to advance. A fresh membrane frame is required for each test. Although the tape drive functioned throughout the experiments, being mechanical, it is succeptible to breakdown.

New generation identification instruments for biological agents would ideally be significantly smaller, have fewer mechanical subsystems and perform assays in a shorter time period. Other useful features would be an absence of membranes or fixed active surfaces. The total assay time was 15 min of which only 3.5 min was allotted to immunochemistry. The remainder was taken up by transporting fluids, filtration, washing and membrane reading. Analytical devices based on microchip platforms would provide many desirable features in new generation instrumentation (15).

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Table I. Limits of Detections Using Standard Curves for Various Trials

Trial #	initial curve	1,5,7,13	2,8,9,10,11	3,4,6,23,24,25	14,15,16	17,18,19,20,21,22
LOD (ng/mL)	33	30	9	27	28	18

The concentrations given in Tables II and III were caclulated from the standard curves indicated above.

Table II. BD Response for Field Trail Samples Collected on LVAS in Trailer

Trial #	Environmental Background Sample				Aerosol Sample			
	Signal (μV/sec)	S/N	alarm*	μg/mL	Signal (μV/sec)	S/N	alarm	μg/mL
1	47±17	1.0	-	0.01	3472±562	62.0	+++	outside curve
3	159±35	4.3	+	0.13	964±225	26.1	+	1.12
4	478±177	12.9	+	0.48	1645±226	44.5	++	2.60
7	68±18	1.1	_	0.04	1402±171	22.6	++	2.81
8	164±19	5.5	+	0.12	1538±389	52.8	++	1.42
10	52±13	1.7	-	0.04	948±226	31.6	+	0.76
11	192±103	6.4	+	0.14	2198±680	73.3	+++	outside curve
12	141±23	2.7	-	0.13	257±50	4.9	+	0.24
13	738±175	16.0	+	1.08	2311±670	50.2	+++	outside curve
14	229±30	3.4	+	0.13	1792±364	26.4	++	1.39
15	86±16	1.3	-	0.02	1662±466	24.4	++	1.26
16	161±29	2.4	-	0.07	885±247	13.0	+	0.62
17**	54±12	1.1	-	0.03	39±14	1.0	-	0.002
18	117±44	2.5	-	0.10	1561±207	33.2	++	1.50
19	1023±262	21.8	++	0.87	1324±645	28.2	++	1.20
20	148±23	3.1	-	0.13	735±161	15.6	+	0.59
21**	155±40	3.3	-	0.14	107±27	2.3	-	0.09
22	622±118	13.2	+	0.49	304±124	6.5	+	0.22
23	111±23	2.8	-	0.07	812±185	20.3	+	0.90
24	765±237	19.1	+	0.84	1729±529	43.2	++	outside curve
25	519±119	14.0	+	0.53	1517±210	41.0	++	outside curve

* - no alarm, + low alarm, ++ medium alarm, +++ high alarm

** *Erwinia herbicola* trials; S/N was the ratio of the signal to the negative control

The signals (μV/sec) were data output from the BD and are the mean and standard deviation of 7 or 8 measurements.; sample concentrations (μg/mL) were obtained from calibration curves. The samples given by Trial # in Tables II & III were from the same field trial dispersal.

Table III. BD Response for Field Trail Samples Collected on L97 and XMX

Trial #	V97 LVAS				XMX Sampler			
	Signal (μV/sec)	S/N	alarm*	μg/mL	Signal (μV/sec)	S/N	alarm	μg/mL
Background	73±11	2.4	-	0.05	43±10	1.4	-	0.03
1					133±33	2.4	-	0.13
2**	78±21	2.6	-	0.05	63±12	2.1	-	0.04
3	440±150	11.9	+	0.43	187±48	5.1	+	0.17
4	594±116	16.1	+	0.62	276±41	7.5	+	0.25
5**	68±14	1.2	-	0.04				
6	258±60	6.5	+	0.23	474±139	11.9	+	0.47
7					74±15	1.2	-	0.05
8	1334±346	44.5	++	1.14	738±159	24.6	+	0.58
9					93±19	3.1	-	0.07
10	1558±409	51.9	++	1.39				
11					451±209	15.0	+	0.34
12	787±182	15.1	+	0.64	127±23	2.4	-	0.11
13					113±23	2.5	-	0.10
14	1378±255	20.3	++	1.01	832±164	12.2	+	0.59
15	1002±247	14.7	++	0.71	208±38	3.1	+	0.11
16	1656±689	24.3	++	1.26	166±23	2.4	+	0.08
17**	130±19	2.8	-	0.11				
18	734±91	15.6	+	0.59	106±19	2.3	-	0.09
19	248±42	5.3	+	0.21	70±19	1.5	-	0.05
20	734±164	15.6	+	0.59	114±25	2.4	-	0.10
21**	112±14	2.4	-	0.10				
Background	71±10	2.4	-	0.05				
22	1848±263	39.3	++	1.93				
23	1357±367	33.9	++	1.81				
24	986±335	24.7	+	1.16				
25	900±135	24.3	+	1.03	752±141	20.3	+	0.82

* - no alarm, + low alarm, ++ medium alarm, +++ high alarm

** *Erwinia Herbicola* trials; S/N was the ratio of the signal to the negative control.

Signals are the mean and standard deviation of 7 or 8 measurements.

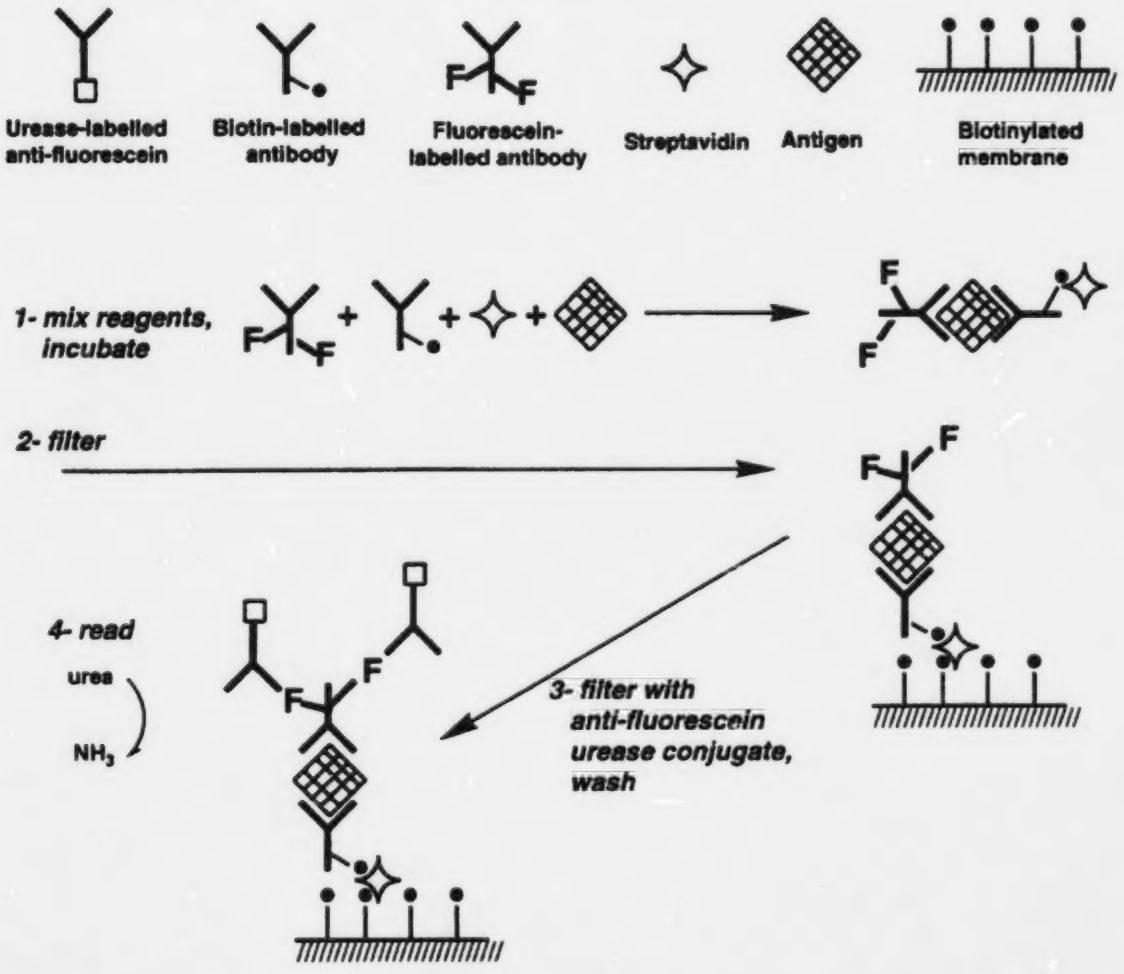

Figure 1: Assay scheme for BioDetector assays of *Bacillus globigii*

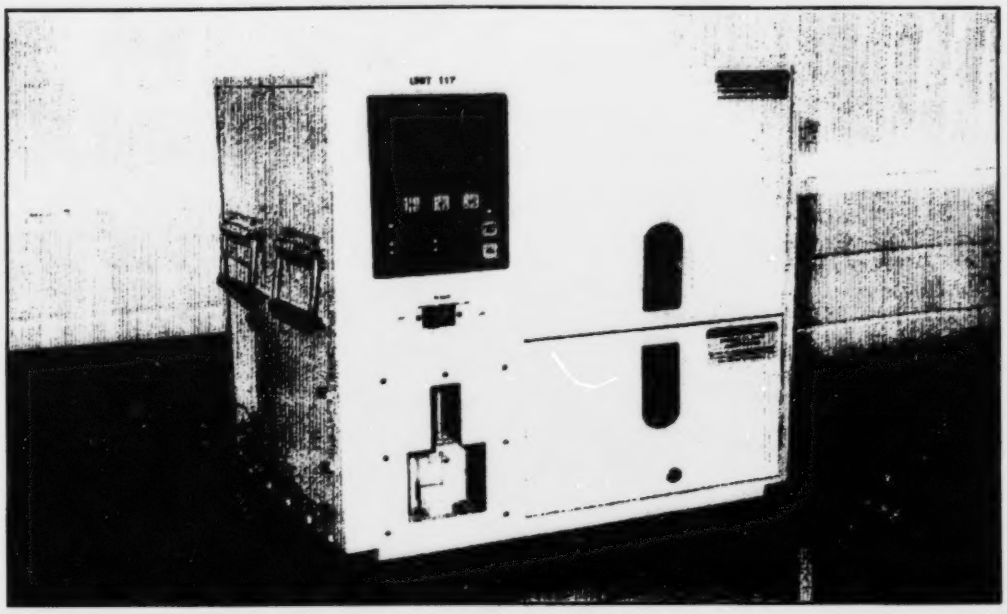

Figure 2: The BioDetector on the bench. Dimensions are 55 cm (h) x 55 cm (w) x 45 cm (d) and weight is 55 kg.

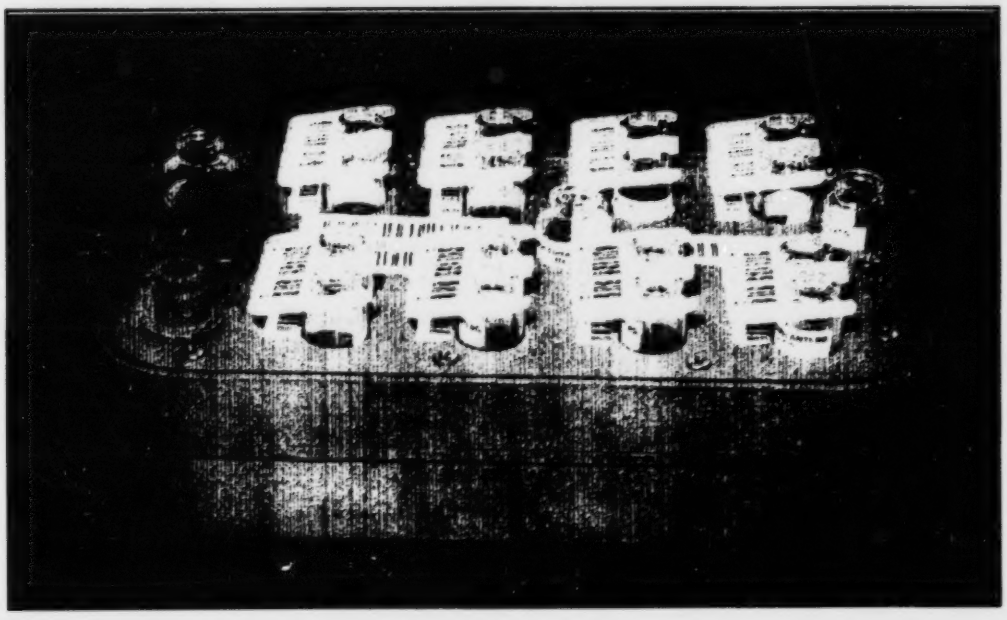

Figure 3: The reagent tray contained sufficient reagents and buffers to analyse 35 samples for eight independent assays. In the work presented here reagents for BG were used with each channel. Barcode tags were attached to the reagent vials to register the contents of the reagent vials with the instrument software.

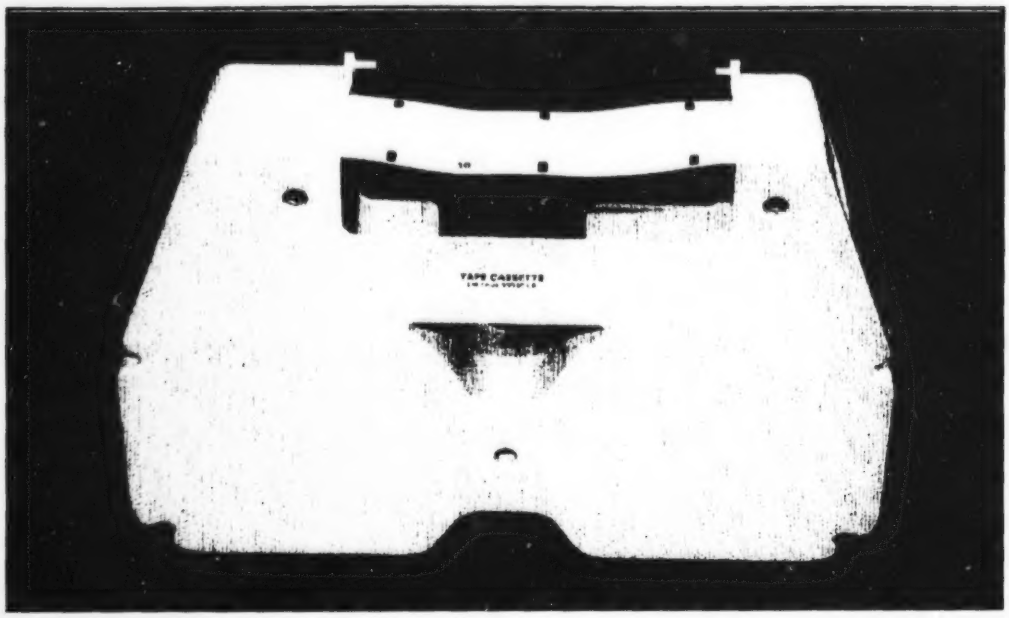

Figure 4: The membrane cassette contained approximately 40 frames of biotin-embedded nitrocellulose membrane. Immunoassay reaction products were captured on the nitrocellulose membrane as indicated by the scheme in Figure 1. The frames were advanced between sample by a mechanical tape drive. Positioning of the frames with respect to the LAPS reader was accomplished by internal optical devices that sensed the position of the hole cut into the PVC tape backing as shown in the photograph.

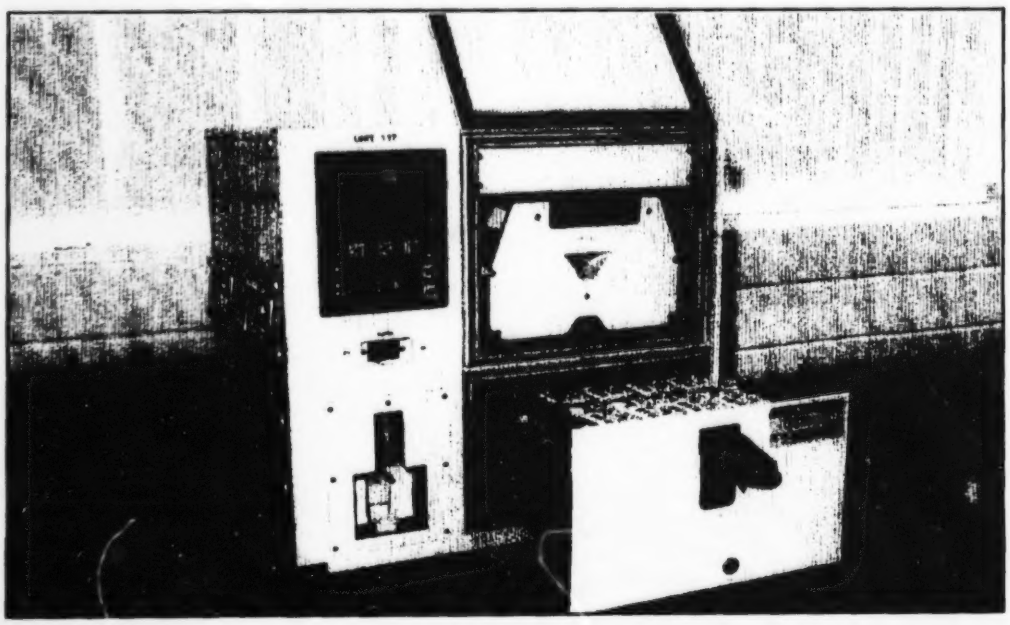

Figure 5: Exposed view of the BioDetector. Upper compartment contains the membrane cassette; lower compartment contains the reagent tray.

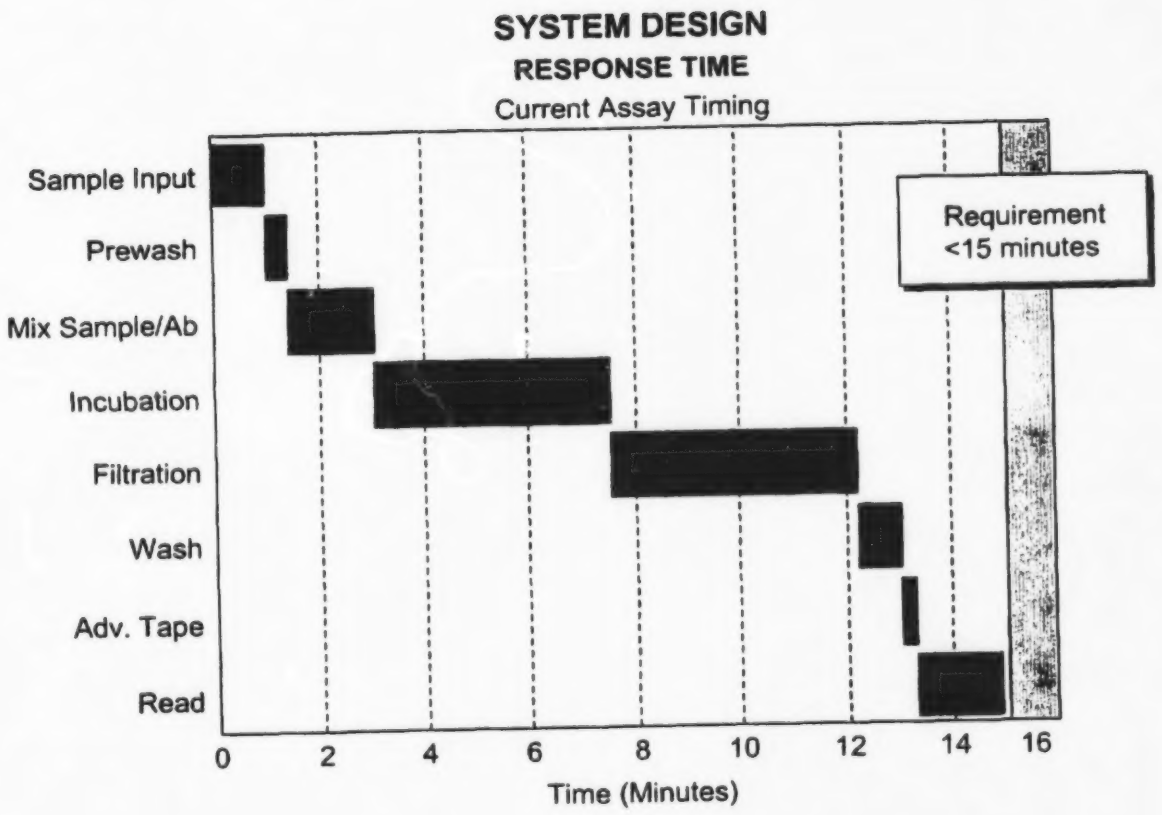

Figure 6: Timing sequence of the BD. Total assay time was less that 15 min.

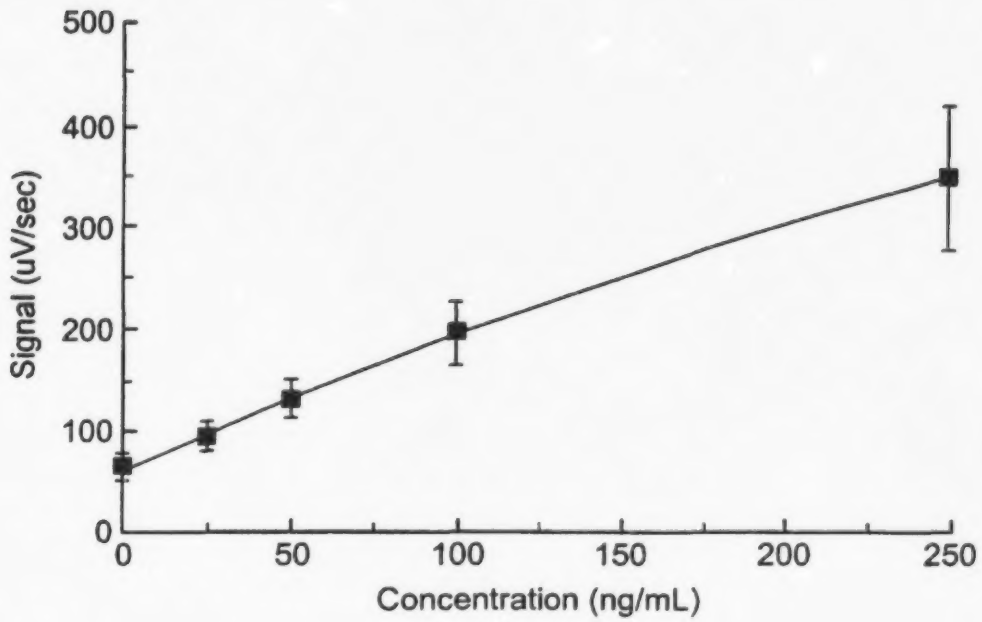

Figure 7: Standard curve of the BD response to *bacillus globigii* in the low concentration regime.

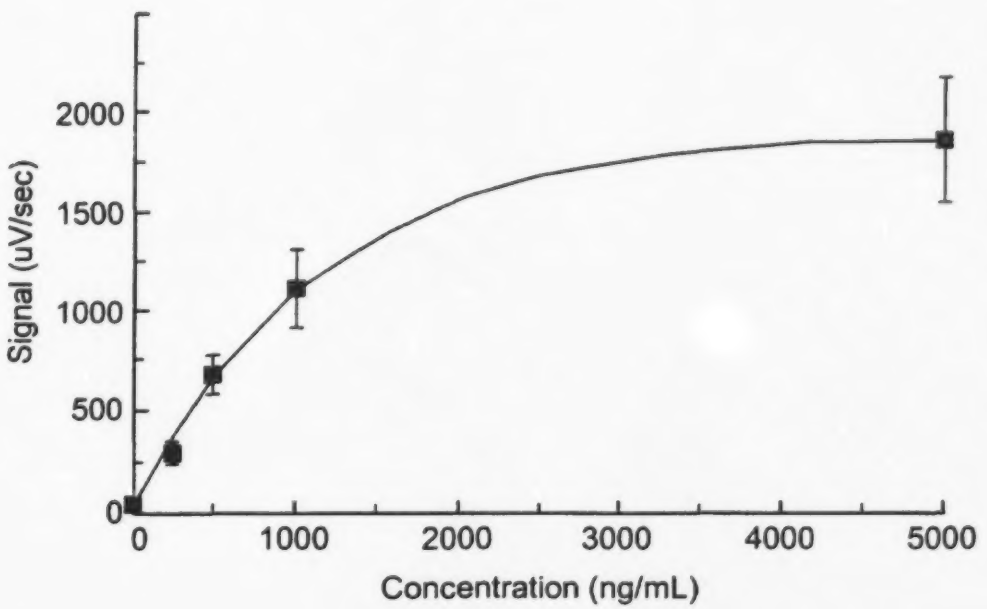

Figure 8: Standard curve of the BD response to *bacillus globigii* in the high concentration regime.